AMRESCO Directions for Use

Product Line: **Detection**

Product Code: **K918-KIT – Gram Staining Kit**

Procedure:

1. Heat-fix a smear of a bacterium as follows:

   Place a drop of water on a clean slide and aseptically transfer a small amount of the bacterium to the drop of water and mix. Using a loop, spread the mixture over a large surface of the slide to form a thin film. Allow this thin suspension to completely air dry. Pass the slide, bacteria side up, through the flame of a Bunsen burner three (3) or four (4) times to heat-fix.

2. Replace the black caps of each bottle of Gram reagent with the spout caps provided in the kit.

3. Flood the area of bacteria with Gram Crystal Violet. Let the Gram Crystal Violet stand for about sixty (60) seconds. Wash the slide for five (5) seconds with slow-running water. The specimen should appear blue-violet when observed with the naked eye.

4. Cover the bacteria with the Gram Iodine. Let this stand approximately one (1) minute as well. Rinse the slide with water for five (5) seconds and immediately proceed to the next step. At this point, the specimen should still be blue-violet.

5. Add the Decolorizer Solution drop-wise until the blue-violet color is not longer emitted from the specimen. Rinse the slide with water for five (5) seconds with slow-running water.

6. Cover the bacteria with the Gram Safranin. Let this stand for approximately one (1) minute to allow the bacteria to incorporate the Gram Safranin. Gram-positive cells will remain blue-violet in appearance. Gram-negative bacteria, however, take on a pink color and are easily distinguishable from the Gram-positives. Again, rinse with water for five (5) seconds to remove any excess dye.

7. Blot the slide gently with bibulous paper or allow it to air-dry before viewing it under a bright field microscope using an oil-immersion objective. (Note: Do not use a phase-contrast objective.) A drop of oil can be placed directly on the slide.

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